

Claims

1. A process for the specific oxygenation of an organic compound by contacting this compound with molecular oxygen in the presence of a cell-free microbial monooxygenase.
- 5 2. The process of claim 1, wherein the oxygenation reaction comprises one of the reactions of Figure 2.
3. The process of claims 1-2, wherein the monooxygenase is supplied with necessary reduction equivalents by a reduced metal complex especially
10 comprising Cyclopentadienyl bipyridyl rhodium complexes wherein one or both of the pyridine rings can be substituted.
4. The process of claim 3, wherein the monooxygenase component is regenerated directly (Figure 1 and/or Figure 4) or at any point of its electron
15 transport chain (e.g. replacing the NAD(P)H:acceptor oxidoreductase).
5. The process of claims 3-4, wherein the reduced complex is *in situ* regenerated either electrochemically or chemically.
- 20 6. The process of claims 3-4, wherein the reduction equivalents can be derived chemically either from formate or alcohols.
7. The process of claims 3-4, wherein the electrochemical supply with reduction equivalents is achieved from a cathode with a electrochemical
25 potential in the range of -450 to -900 mV (vs. Ag/AgCl_{sat.}).
8. The process of claim 7, wherein the enzyme is separated from the electrochemical cell via compartmentation (Figure 6).
- 30 9. The process of claim 8, wherein the monooxygenase is retained from the electrochemical cell either by immobilization to a solid matrix within a plugged-flow-reactor or within a continuously stirred tank reactor or in an

enzyme-membrane reactor.

10. The process of claim 7-9, wherein the oxygen supply for the biocatalyst is controllable so that at the efflux of the biocatalyst compartment the oxygen
5 saturation is minimized.

11. The process of any one of claims 1-10 wherein a second phase, either solid or liquid is used during the reaction to extract any product as soon as it is formed to prevent product decay on in order to act as substrate reservoir.
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12. The process of claim 11, wherein to phase contact is established either by direct contact (e.g. emulsion) or with constant phase separation (e.g. by a hollow-fiber module).

13. The process of any of claims 1-12, wherein inactivation of either biocatalyst or metal complex is prevented either by utilization of nucleophilic buffer additives (e.g. NH_3 or TRIS) or by immobilization of the metal complex.
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14. The process of claims 3-12, wherein the monooxygenase can contain FAD, or FMN, or heme-iron or non-heme-iron or any other metal as cofactor or prosthetic group.
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15. The process of claim 14, wherein the monooxygenase can be used for oxidation reactions such as:

25 a) oxidation of saturated, unsaturated aliphatic or aromatic carbon atoms, especially via hydroxylation, epoxidation or Baeyer-Villiger oxidation (insertion in C-C-bonds);

b) oxidation of heteroatoms from the groups III, V, VI, and VII within the substrate, especially oxidation of boron, nitrogen, phosphorous, sulfur,
30 selenium, bromine, and iodine.

16. A process for *in situ* generation of hydrogen peroxide coupled to an enzymatic reaction, preferably to a monooxygenase or a peroxidase for the oxidation of organic compounds.
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17. The process of claim 16, wherein the reduced metal complex acts on alloxazine moieties such as FAD or FMN in the presence of molecular oxygen to form hydrogen peroxide.
- 5 18. The process of claims 16-17, wherein the reaction conditions are according to claims 4-17.
19. A process of *in situ* regeneration of NAD(P)⁺ from NAD(P)H.
- 10 20. The process of claim 19, wherein the oxidized coenzyme is consumed as cosubstrate by a dehydrogenase within an oxidation reaction.
21. The process of claims 19-20, wherein a inorganic mediator such as [Cp*Rh(bpy)(H₂O)]²⁺ is used to catalyze the transhydrogenation reaction from
15 NAD(P)H to an alloxazine based acceptor (such as FAD, FMN).
22. The process of claims 19-21, wherein the reduced acceptor is reoxidized either by molecular oxygen or by anodic oxidation.
- 20 23. The process of claim 22, wherein the accumulation of hydrogen peroxide is prevented either by chemical or enzymatic dismutation.